# RP-HPLC Estimation of Imipramine Hydrochloride and Diazepam in Tablets

D. SRIKANTHA AND R. R. RAJU*
Department of Chemistry, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur-522 510, India



**A simple and rapid reversed phase-high performance liquid chromatographic method was developed for simultaneous determination of imipramine hydrochloride and diazepam in pharmaceutical formulations. The elution was done in isocratic mode utilizing a mobile phase consisting of methanol:water:0.1M sodium acetate (30:50:20 v/v/v) on Chromosil C18 column with a flow rate of 1.0 ml/min and with detection at 243 nm. The measured retention time was 3.33±0.02 min for imipramine hydrochloride and 4.64±0.02 min for diazepam. Linearity was measured in the range 25-150 µg/ml for imipramine hydrochloride ($r^2$=0.999) and in the range 5-30 µg/ml for diazepam ($r^2$=0.9994), respectively. The limits of detection and quantitation were 0.03 and 0.1 µg/ml for imipramine hydrochloride and 0.02 and 0.07 µg/ml for diazepam. Satisfactory validation was also obtained from recovery (100.95-101.52% for imipramine hydrochloride and 99.47-100.33% for diazepam) studies, intraday and interday precision (<2%) and robustness results. The reported method was the first study of these drugs in combination and could be employed for routine quantitative determination of imipramine hydrochloride and diazepam in tablets.**

**Key words: Imipramine HCL, Diazepam, RP-HPLC, UV detection, Tablet dosage form**

Imipramine hydrochloride (IPM) is a tricyclic antidepressant[1]. Its chemical name is 10,l1-dihydro-5Hdibenz[b,f]azepine-5-(dimethylaminopropy1) hydrochloride. The molecular formula is $C_{19}H_{24}N_2$. HCl and the molecular mass is 316.9 g/mol. It is officially recognized in Indian Pharmacopoeia (IP)[1], British Pharmacopoeia (BP)[2] and the United States Pharmacopoeia (USP)[3]. Diazepam (DZM) an antianxiety, antiepileptic drug with the IUPAC name, 7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one. The molecular formula of diazepam is $C_{16}H_{13}ClN_2O$ and the molecular weight is 284.7 g/mol. It is also officially recognized in IP[1], BP[2] and USP[3].

IPM was studied as a single drug and in combination with other drugs[4-6]. Different methods like second-order derivative spectrophotometry[5], chemometric method[6], spectrophotometry[7,8], high performance liquid chromatography (HPLC)[4,9,10], fluorescence polarization immunoassay[10], HPLC-diode array detection (HPLC-DAD)[11], surface ionization organic mass spectrometry[12] and HPLC tandem mass spectrometry (HPLC-MS/MS)[13] have been employed to measure IPM. It was also determined in human serum[4,9,13] and urine[4,9].

Diazepam has been studied separately, in combination with other benzodiazepines and other drugs[14-20] and its metabolites[21]. It was studied in whole blood[16], plasma[17,18], urine, hair and oral fluids[19,22] and in pharmaceutical form[23-26]. These studies were carried out using analytical methods such as second-derivative spectrophotometry[5], chromatography[15], HPLC[16-18,26], LC-MS/MS[18,19], HPLC electrospray tandem mass spectrometry[20], capillary electrophoresis

***Address for correspondence**
E-mail: rrraju1@gmail.com



**Accepted 18 April 2015**
**Revised 08 December 2014**
**Received 02 November 2013**
**Indian J Pharm Sci 2015;77(3):343-347**

and HPLC-electrospray ionization mass spectrometry (HPLC-ESI-MS/MS)[21], gas chromatography-negative ion chemical ionization mass spectrometry[22], spectrophotometry[23] fluorimetry[24], differential pulse polarographic determination[25] and capillary electrophoresis[26].

IPM and DZM combination is available as Imipam (La Pharma), Imidep (Sunrise Remedies), Imilor (Intra Doxis) and Imizonic plus (Acme) and many others. The motivation for the present work is that benzodiazepines and tricyclic antidepressants are often administered to patients in combination for the treatment of anxiety and depression related disorders. This clinical use necessitates quantitative estimation of these drugs in combination using a simple and rapid method.

In this paper, the development of a method for quantitative and simultaneous estimation of imipramine hydrochloride and diazepam in combination is reported. The developed method is validated by measuring linearity, limit of detection (LOD), limit of quantitation (LOQ), precision, accuracy, robustness, recovery, ruggedness and specificity based on ICH guidelines[27]. To the best of our knowledge, this is the first work reporting simultaneous determination of IPM and DZM drug combination by RP-HPLC method using ultraviolet (UV) detection.

The chemicals methanol and water (pH between 5 and 8) were of HPLC grade and procured from Merck Limited, Mumbai. Analytical grade sodium acetate and acetic acid were used (Merck Limited, Mumbai). Formulations of the drug were purchased from a local pharmacy. For filtering the prepared solutions 0.45 µm nylon membrane filter paper (Ultipore, Mumbai) was used.

A Peak HPLC system operated in isocratic mode was utilized for the presented investigations. A Chromosil C18 column (250×4.6 mm, 5 µm particles) was used as a stationary phase. The HPLC system was equipped with a LC 20AT pump and a variable wavelength programmable UV/Vis detector (Shimadzu, SPD-10Avp). The samples were injected with a 20 µl Hamilton syringe. The degassing of the mobile phase was done with an ultrasonic bath sonicator and a digital electronic balance was used for weighing the materials. Chromatograms were recorded and integrated by Peak LC 7000 software (MSP Lab instruments, India). Analysis of the data was done with Microsoft Excel software. The detection wavelength of IPM and DZM was determined with an ultraviolet UV/Vis spectrophotometer (Techcomp, UV 2301) provided with Hitachi software.

To prepare the stock solution, 10 mg of IPM and DZM was weighed and dissolved in 10 ml of methanol separately in a 10 ml volumetric flask. Then the drug was sonicated for 2 min to dissolve completely. After cooling, the drug was filtered through 0.45 µm nylon membrane filter paper. A 1000 µg/ml solution was prepared, from this 2 ml was further diluted to 20 ml to get a stock concentration of 100 µg/ml solution. Required concentrations were prepared by selective dilution from the standard solution.

The RP-HPLC method with UV detection was started with the development of the mobile phase composition, flow rate, wavelength and pH. These were optimized for sharper peaks with less asymmetry and for good resolution of IPM and DZM drugs. Initially, mobile phase volume ratio was developed. Methanol and water were tested separately and in combination as a mobile phase. Further, sodium acetate was added to methanol and water, which increased the pH of the mobile phase. pH was decreased to 3.8 by using dilute acetic acid. After several chromatographic runs, methanol:water:0.1 M sodium acetate (30:50:20 v/v/v) showed better peak symmetry, good signal to noise (S/N) ratio and well separated chromatographic peaks. Similarly, different pump pressures were tried and the pressure was set to 12.5 MPa. Optimum flow rate was determined by running the solutions at different flow rates and was set to 1.0 ml/min. The elution runtime was limited to ten minutes after checking for the interference and influence of the excipients.

The optimum chromatographic conditions obtained during the method development were: mobile phase-methanol:water:0.1M sodium acetate (30:50:20 v/v/v); detection wavelength-243 nm; stationary phase-Chromosil C18 column (250×4.6 mm, 5µm); pH of the mobile phase-3.8 adjusted with diluted acetic acid; active pharmaceutical ingredient

concentration-75 µg/ml (IPM) and 15 µg/ml (DZM); flow rate-1.0 ml/min; pump pressure-12.5±0.5 MPa and runtime-10 min.

The spectra of diluted solutions of IPM and DZM in methanol were recorded on an UV spectrophotometer by scanning the wavelength from 200 to 400 nm. The peak of maximum absorbance for both IPM and DZM showed a wavelength of 243 nm (fig. 1). For simultaneous analysis of the two drugs; first the single drugs were scanned individually and then maximum absorption wavelength was chosen from the overlay spectra. By complete separation of IPM and DZM the specificity of the developed RP-HPLC method was validated with retention time, tailing factor and resolution. The measured peaks for both the drugs were sharper and were well separated. The chromatogram of IPM and DZM from standard solution was shown in fig. 2. The retention time was 3.33±0.02 for IPM and 4.64±0.02 min for DZM for 10 min runtime, respectively. The tailing factors of IPM and DZM were 0.79 and 1.03 and these values fall in the range of recommended values. Furthermore, the theoretical plates of IPM (12184) and DZM (2862) were well above the recommended values.

The linearity measurements were carried out for six concentrations in the range 25-150 µg/ml in 25 µg/ml steps for IPM and in the range 5-30 µg/ml in 5 µg/ml steps for DZM, respectively. These measurements were fitted with a linear regression of the form y=ax+b and the values of regression parameters for the curves were $r^2$=0.999 for IPM and $r^2$=0.9994 for DZM. All the linear regression parameters were statistically significant.

The precision studies of the two drugs IPM (75 µg/ml) and DZM (15 µg/ml) were done by intraday and interday precision (for three successive days) measurements. The data obtained from intraday precision and interday precision was given in Table 1. The peak areas were expressed in term of mean normalized area of six samples. Table 1 gives standard deviation (SD) and relative standard deviation (RSD) along with the recommended values of these parameters. The RSD of intraday analysis of all the samples was 0.31 and 0.61, and interday analysis was 0.85 and 0.99 for IPM and DZM, respectively. This was less than 1% indicating the method was precise. Ruggedness of IPM (75 µg/ml) and DZM (15 µg/ml) was checked by two different analysts. In total six samples were analyzed and the results were given in Table 1. The RSD was less than 2% indicating the ruggedness of the method.

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated from the calibration curves of IPM and DZM drugs. These were determined from the sensitivity during linearity measurements. For IPM, LOD was 0.03 µg/ml and LOQ was 0.1 µg/ml. For DZM, LOD was 0.02 µg/ml and LOQ was 0.07 µg/ml.

The developed method was validated for robustness by changing the mobile phase volume ratio, flow rate and wavelength from the optimal chromatographic parameters. The percentage change of peak areas was calculated for the above three parameters. The observed change was less than 2% from the standard value (Table 2). The results indicate that the developed method was robust.

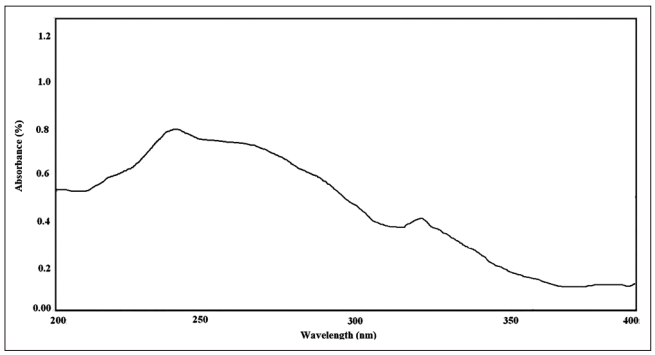


**Fig. 1: Absorption spectrum of IPM and DZM.**
**Absorption spectrum of imipramine hydrochloride (IPM) and diazepam (DZM). Absorbance as a function of wavelength. The maximum absorption was observed at 243 nm.**

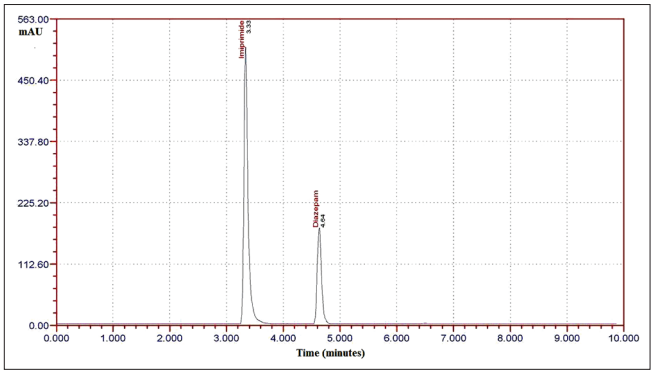


**Fig. 2: Chromatogram of IPM and DZM.**
**Chromatogram of imipramine hydrochloride (IPM) and diazepam (DZM), whose retention times were 3.33 and 4.64 min, respectively.**

**TABLE 1: VALIDATION PARAMETERS**

| Parameter | IPM | | DZM | | Recommended RSD |
|---|---|---|---|---|---|
| | Normalized area mean±SD (*n*=6) | % RSD | Normalized area mean±SD (*n*=6) | % RSD | |
| Intraday precision | 1.01±0.02 | 1.79 | 0.97±0.02 | 1.62 | <2 % |
| Intraday precision | 1.01±0.01 | 1.1 | 1.014±0.01 | 1.23 | <2 % |
| Ruggedness | 0.98±0.01 | 1.22 | 0.99±0.01 | 1.24 | <2 % |

Results of validation parameters such as intraday, interday precision and ruggedness for IPM (imipramine hydrochloride 75 µg/ml) and DZM (diazepam, 15 µg/ml). RSD is relative standard deviation. RSD: relative standard deviation, SD: standard deviation

**TABLE 2: ROBUSTNESS STUDIES OF IPM AND DZM**

| Parameter | Value | IPM[a] | | DZM[b] | |
|---|---|---|---|---|---|
| | | Area (mAU) | % change | Area (mAU) | % change |
| Standard | | 535134 | - | 248816 | - |
| Mobile phase | 35:45:20 | 535841 | 0.69 | 249418 | 0.24 |
| Methanol:water:0.1M sodium acetate | 25:55:20 | 532425 | 0.13 | 249993 | 0.47 |
| pH (adjusted with dil. acetic acid) | 3.7 | 532173 | −0.50 | 250394 | 0.63 |
| | 3.9 | 525043 | −0.55 | 249562 | 0.29 |
| Wavelength (nm) | 241 | 542416 | −1.8 | 249724 | 0.36 |
| | 245 | 538835 | 1.3 | 253108 | 1.72 |

Results of robustness studies of imipramine hydrochloride (IPM) and diazepam (DZM) with variation of parameters such as, mobile phase, volume ratio, pH and detection wavelength. [a]At 75 µg/ml and [b]at 15 µg/ml. IPM: imipramine hydrochloride, DZM: diazepam

**TABLE 3: RECOVERY STUDIES OF SPIKED SAMPLES OF IPM AND DZM**

| Analyte | Recovery (%) | Target Conc. (µg/ml) | Spiked Conc. (µg/ml) | Final Conc. (µg/ml) | Conc. obtained (µg/ml) Mean±SD | RSD | % Recovery Mean±SD | % Error[a] |
|---|---|---|---|---|---|---|---|---|
| IPM | 50 | 50 | 25 | 75 | 76.14±0.51 | 0.67 | 101.52±0.8 | 0.38 |
| | 100 | 50 | 50 | 100 | 100.59±0.9 | 0.9 | 100.59±0.91 | 0.52 |
| | 150 | 50 | 75 | 125 | 126.19±1.2 | 0.98 | 100.95±0.99 | 0.57 |
| DZM | 50 | 10 | 5 | 15 | 14.94±0.25 | 1.7 | 99.63±1.67 | 0.98 |
| | 100 | 10 | 10 | 20 | 19.89±0.20 | 1.0 | 99.47±1.03 | 0.58 |
| | 150 | 10 | 15 | 25 | 25.17±0.31 | 1.2 | 100.33±1.23 | 0.69 |

Results of recovery studies with spiked samples of imipramine hydrochloride (IPM) and diazepam (DZM) using standard addition method. [a]% error is RSD/√n, no of trials n=3. RSD: relative standard deviation, SD: standard deviation

The accuracy of the proposed method was verified using standard addition technique by adding known amount of standard to samples (spiked samples). Three different percentage determinations (50, 100 and 150%) were used for the recovery studies. For each percentage level the analysis was repeated three times (*n*=3) for IPM and DZM. The recovery percentage was compared with the actual amount. The results were presented in Table 3 as the mean concentrations and their standard deviation (SD) for each percentage level. The relative standard deviation (RSD) or the coefficient of variation (CV) and calculated percentage error was also given (Table 3). The good recovery of the two drugs IPM and DZM in the range of 100.59-101.52% and 99.47-100.33% satisfy the ICH guidelines[27].

Formulation assay was performed on commercial Imipam (La Pharma) tablets. The procedure was repeated two times, separately. Twenty tablets were powdered and weighed. The powdered drug equal to 1 mg was taken and dissolved in 10 ml of methanol. From a concentration of 100 µg/ml solution, 75 and 15 µg/ml were prepared. From formulation assay studies 74.6 and 14.9 µg/ml were found, respectively. The assay results of the two drugs were expressed as percentage of label claims. The recovery percentages were 99.4% for IPM and 99.9% for DZM that were in good agreement within the 90 to 100% of the label claim. The chromatogram peaks of the IPM and DZM drugs were predominant in the drug sample with negligible interference from excipients, normally present in the tablets.

In summary, the described RP-HPLC method in this paper was simple and sensitive, fast and specific to carry out. The reported method facilitates for simultaneous determination of imipramine hydrochloride and diazepam with good resolution and sharper chromatographic peaks within a run time of

10 min. The presented results of validation parameters intraday and interday were precise and recovery results were accurate. They were established by statistical parameters and satisfy the ICH guidelines. Hence, this method can be used for routine quantitative analysis of these drugs in combination and in pharmaceutical preparations in industry and laboratories.

**Financial support and sponsorship:**
Nil.

**Conflict of interest:**
There are no conflicts of interest.

## REFERENCES

1. Indian Pharmacopoeia. Vol. 2. Gaziabad: The Indian Pharmacopoeia Commission; 2010. p. 1488, 1912.
2. British Pharmacopoeia, Vol. 3. 7th ed of the European Pharmacopoeia, 2013; CD-ROM version 17.0.
3. United States Pharmacopoeia, The United States Pharmacopeia, USP 30/The National Formulary, NF 25; Rockville, MD: U.S. Pharmacopeial Convention, Inc; 2007. p. 2336, 1912.
4. Chen AG, Wing YK, Chiu H, Lee S, Chen CN, Chan K. Simultaneous determination of imipramine, desipramine and their 2- and 10-hydroxylated metabolites in human plasma and urine by high-performance liquid chromatography. J Chromatogr B Biomed Sci 1997;693:153-8.
5. Umapathi P, Parimoo P. Second-order derivative spectrophotometric assay for imipramine hydrochloride and diazepam in pure admixtures and in dosage forms. J Pharm Biomed Anal 1995;13:1003-9.
6. Markopoulou CK, Malliou ET, Koundourellis JE. Application of two chemometric methods for the determination of imipramine, amitriptyline and perphenazine in content uniformity and drug dissolution studies. J Pharm Biomed Anal 2005;37:249-58.
7. Ullah Khan I, Aman T, Kazi AA, Azizuddin R. Spectrophotometric Determination of Imipramine-HCl in Pure and Pharmaceutical Preparations. Anal Lett 1999;32:2061-9.
8. El-Gendy AE, El-Bardicyy MG, Loutfy HM, El-Tarras MF. Flow injection analysis of pharmaceutical compounds. VI. determination of some central nervous system acting drugs by UV-spectrophotometric detection. Spectro Lett 1993;26:1649-60.
9. Nielson KK, Brosen K. High-performance liquid chromatography of imipramine and six metabolites in human plasma and urine. J Chromatogr 1993;612:87-94.
10. Hacket LP, Dusei LJ, Ilett KF. A comparison of high performance liquid chromatography and fluorescence polarization immunoassay of therapeutic drug monitoring of tricyclic antidepressants. Ther Drug Monit 1998;20:30-4.
11. Madej K, Parczewski A, Kała M. HPLC/DAD screening method for selected antipsychotropic drugs in blood. Toxicol Mech Methods 2003;13:121-7.
12. Fujii T, Kurihara Y, Arimoto H, Mitsutsuka Y. Surface ionization organic mass spectrometry of imipramine, desipramine, clomipramine and lidocaine. Anal Chem 1994;66:1884-9.
13. Kirchherr H, Kuhn-Velten WN. Quantitative determination of forty-eight antidepressants and antipsychotics in human serum by HPLC tandem mass spectrometry: A multi-level, single-sample approach. J Chromatogr B Analyt Technol Biomed Life Sci 2006;843:100-13.
14. Drummer OH. Review methods for the measurement of benzodiazepines in biological samples. J Chromatogr B Biomed Sci Appl 1998;713:201-25.
15. Sioufi A, Dubois JP. Chromatography of Benzodiazepines. J Chromatogr 1990;531:459-80.
16. Bugey A and Staub C. Rapid analysis of benzodiazepines in whole blood by high-performance liquid chromatography: Use of monolithic column. J Pharm Biomed Anal 2004;35:555-62.
17. Pistos C, Stewart JT. Direct injection HPLC method for the determination of selected benzodiazepines in plasma using a Hisep column. J Pharm Biomed Anal 2003;33:1135-42.
18. Abbara C, Bardot I, Cailleux A, Lallement G, Le Bouil A, Tureant A, *et al*. High performance liquid chromatography coupled with electrospray tandem mass spectrometry (LC/MS/MS) method for simultaneous determination of diazepam, atropine and pralidoxime in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci 2008;874:42-50.
19. Laloup M, Fernandez Mdel M, Wood M, Maes V, De Boeck G, Vanbeckvoort Y, *et al*. Detection of diazepam in urine, hair and preserved oral fluid samples with LC-MS-MS after single and repeated administration of Myolastan® and Valium®. Anal Bioanal Chem 2007;388:1545-56.
20. Kleinschnitz M, Herderich M, Schreier P. Determination of 1,4-benzodiazepines by high performance liquid chromatography electrospray tandem mass spectrometry. J Chromatogr B Biomed Appl 1996;676:61-7.
21. McClean S, O Kane E, Hillis J, Smyth WF. Determination of 1,4-benzodiazepines and their metabolites by capillary electrophoresis and high-performance liquid chromatography using ultraviolet and electrospray ionization mass spectrometry. J Chromatogr A 1999;838:273-91.
22. Cirimele V, Kintz P, Ludes B. Screening for forensically relevant benzodiazepines in human hair by gas chromatography-negative ion chemical ionization-mass spectrometry. J Chromatogr B Biomed Sci Appl 1997;700:119-29.
23. Sadeghi S, Takjoo R, Haghgoo S. Quantitative determination of diazepam in pharmaceutical preparation by using a new extractive-spectrophotometric method. Anal Lett 2002;35:2119-31.
24. Patel RB, Patel AA, Patel SK, Patel SB, Manakiwala SC. A fluorimetric method for the estimation of diazepam. Indian J Pharm Sci 1988;50:319-20.
25. Sreedhar NY, Reddy SJ, Reddy SJ. Differential pulse polarographic determination of diazepam in pharmaceutical formulations. Indian J Pharm Sci 1992;54:22-4.
26. Aurora-Prado MS, Steppe M, Tavares MF, Kedor-Hackmann ER, Santoro MI. Comparison of capillary electrophoresis and reversed-phase liquid chromatography methodologies for determination of diazepam in pharmaceutical tablets. J Pharm Biomed Anal 2005;37:273-9.
27. International Conference on Harmonization. ICH guidelines on validation of analytical procedures: Text and methodology Q2 (R1), Geneva: Expert Working Group (Quality); 2005. p. 1-8.